

**CLAIMS**

1. A method of generating an immune response to a target polypeptide in an animal in which an aqueous liposomal composition is administered subcutaneously or intramuscularly to the animal, the composition comprising liposomes suspended in an aqueous liquid having diameters in the range 100 to 2000 nm and comprising a lipid bilayer and an aqueous intravesicular space, the lipid bilayer being formed of liposome forming components including at least one cationically charged component in an amount such that the liposome forming components have an overall cationic charge, and the aqueous intravesicular space comprising polynucleotide operatively encoding said target polypeptide, whereby the said polynucleotide is delivered to and is expressed in target cells, to form target polypeptide and an immune response to the target polypeptide follows.

2. A method according to claim 1 in which the polynucleotide is double stranded DNA.

3. A method according to claim 2 in which the polynucleotide is in the form of a plasmid including promoter and, optionally, ribosome binding sequences.

4. A method according to claim 1 in which the polynucleotide is RNA.

5. A method according to claim 1 in which the immunogenic polypeptide comprises an antigen or fragment of an antigen of an infectious microbe.

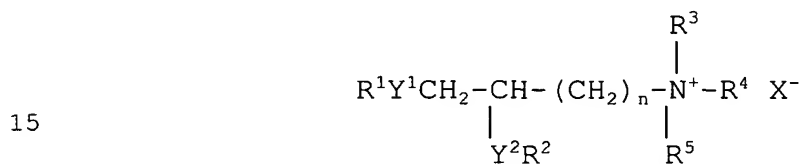
6. A method according to claim 1 comprising the preliminary step of providing said aqueous liposomal composition by a process in which an aqueous suspension of empty liposomes formed from the liposome forming components is provided, the aqueous suspension of empty liposomes is mixed with said polynucleotide to form a mixed suspension, the mixed suspension is dehydrated to form a dehydrated mixture, and the dehydrated mixture is rehydrated in

aqueous composition to form dehydration - rehydration vesicles containing the polynucleotide in the intravesicular space.

7. A method according to claim 6 in which the dehydration-rehydration vesicles are subjected to a micro fluidization step or extrusion.

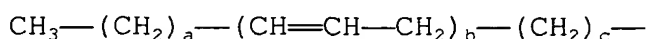
8. A method according to claim 6 in which the dehydration is carried out by lyophilisation.

9. A composition according to claim 1 in which the cationic component is a glyceride having the general formula



or an optical isomer thereof, wherein  $\text{Y}^1$  and  $\text{Y}^2$  are the same or different and are each  $-\text{O}-$  or  $\text{O}-\text{C}(\text{O})-$  wherein the carbonyl carbon is joined to  $\text{R}^1$  of  $\text{R}^2$  as the case may be;  $\text{R}^1$  and  $\text{R}^2$  are independently an alkyl, alkenyl, or alkynyl group of 6 to 24 carbon atoms,  $\text{R}^3$ ,  $\text{R}^4$  and  $\text{R}^5$  are independently hydrogen, alkyl of 1 to 8 carbon atoms, aryl or aralkyl of 6 to 11 carbon atoms; alternatively two or three of  $\text{R}^3$ ,  $\text{R}^4$  and  $\text{R}^5$  are combined with the positively charged nitrogen atom to form a cyclic structure having from 5 to 8 atoms, where, in addition to the positively charged nitrogen atom, the atoms in the structure are carbon atoms and can include one oxygen, nitrogen or sulfur atom;  $n$  is 1 to 8; and  $\text{X}$  is an anion.

10. A method according to claim 9 in which  $\text{R}^1$  and  $\text{R}^2$  individually have from 0 to 6 sites of unsaturation, and have the structure



wherein the sum of  $a$  and  $c$  is from 1 to 23; and  $b$  is 0 to 6.

11. A method according to claim 1 in which the cationic component is selected from the group consisting of DOTAP, BisHOP, DC-Chol and stearylamine.

12. A composition according to claim 1 in which the  
5 liposome forming components include a phosphatidyl ethanolamine.

13. A method according to claim 1 in which the mean diameter of the liposomes in the said aqueous liposomal composition is in the range 200 to 500 nm.

10 14. A method according to claim 1 in which said aqueous liposomal composition comprises 0.1 to 10 µg of polynucleotide per mg liposome forming components.

15 15. A method according to claim 1 in which the method is vaccination to immunise against infective microbes or a cancer cell.

16. A method according to claim 1 in which the composition is administered by intramuscularly.

17. A process for forming an aqueous suspension of liposomes having diameters in the range 100 to 2000 nm  
20 comprising the steps:

a) providing an aqueous suspension of small unilamellar vesicles formed from liposome selected from the group consisting of lipids, cholesterol and non-ionic and cationic surface active agents including at least one  
25 cationically charged component selected from cationic lipids and cationic surface active agents present in an amount whereby the small unilamellar vesicles have an overall cationic charge;

b) adding to the aqueous suspension of small  
30 unilamellar vesicles a polynucleotide operatively encoding an immunogenic polypeptide useful to induce an immune response in an animal to form a mixed suspension;

c) dehydrating the mixed suspension to form a dehydrated mixture; and

d) rehydrating the dehydrated mixture to form an aqueous suspension of dehydration-rehydration vesicles containing said nucleic acid in the intravesicular space thereof; and

5 e) optionally subjecting the aqueous suspension of dehydration-rehydration vesicles to a further step of microfluidisation whereby the said aqueous suspension of liposomes is produced.

10 18. A process according to claim 17 comprising the further step of subjecting the suspension of dehydration-rehydration vesicles to a separate step in which non-entrapped polynucleotide is separated from the aqueous suspension of dehydration-rehydration vesicles.

15 19. A process according to claim 18 in which the level of non-entrapped polynucleotide separated from the suspension is in the range 10 to 90% based on polynucleotide added in step (b).

20 20. A process according to claim 19 wherein the said level is in the range 15 to 80%.

21. A process according to claim 17 in which the polynucleotide is double stranded DNA.

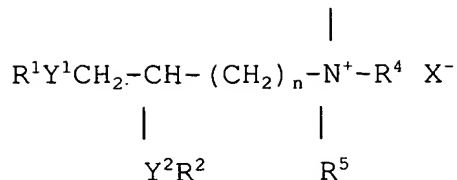
22. A process according to claim 21 in which the polynucleotide is in the form of a plasmid including promoter and, optionally, ribosome binding sequences.

25 23. A process according to claim 17 in which the polynucleotide is RNA.

24. A process according to claim 17 in which the immunogenic polypeptide comprises an antigen or fragment of an antigen of an infectious microbe.

30 25. A process according to claim 17 in which the dehydrating is by lyophilisation.

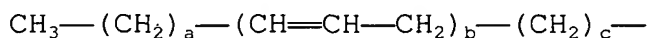
26. A process according to claim 17 in which the cationic component is a glyceride having the general formula



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or an optical isomer thereof, wherein  $Y^1$  and  $Y^2$  are the same or different and are each  $-O-$  or  $O-C(O)-$  wherein the carbonyl carbon is joined to  $R^1$  of  $R^2$  as the case may be;  $R^1$  and  $R^2$  are independently an alkyl, alkenyl, or alkynyl group of 6 to 24 carbon atoms,  $R^3$ ,  $R^4$  and  $R^5$  are independently hydrogen, alkyl of 1 to 8 carbon atoms, aryl or aralkyl of 6 to 11 carbon atoms; alternatively two or three of  $R^3$ ,  $R^4$  and  $R^5$  are combined with the positively charged nitrogen atom to form a cyclic structure having from 5 to 8 atoms, where, in addition to the positively charged nitrogen atom, the atoms in the structure are carbon atoms and can include one oxygen, nitrogen or sulfur atom;  $n$  is 1 to 8; and  $X$  is an anion.

27. A process according to claim 26 in which  $R^1$  and  $R^2$  individually have from 0 to 6 sites of unsaturation, and have the structure



wherein the sum of  $a$  and  $c$  is from 1 to 23; and  $b$  is 0 to 6.

28. A process according to claim 17 in which the cationic component is selected from the group consisting of DOTAP, BISHOP, DC-Chol and stearylamine.

29. A process according to claim 17 in which the liposome forming components include a phosphatidyl ethanolamine.

30. A process according to claim 17 in which the small unilamellar vesicles in step (a) have a diameter in the range 100 to 400nm.

31. A process according to claim 17 in which the dehydration-rehydration vesicles produced in step d) have diameters in the range 200 to 2000 nm.

5 32. A process according to claim 17 in which the weight ratio of liposome forming components making up the small unilamellar vesicles in step (a) to the polynucleotide add in step (b) is in the range (50 to 10000):1.

10 33. A method according to claim 1 in which the immune response involves both cell-mediated and humoral responses.

34. A composition for administration to an animal to protect against infection by target infections microbes comprising liposomes having diameters in the range 100 to 2000nm and having lipid-bilayers formed from liposome  
15 forming components selected from the group consisting of glycerides, cholesterol and non-ionic and cationic surface active agents including at least one cationic component selected from cationic surface active agents and cationic lipids in an amount to confer an overall cationic charge on  
20 the liposome forming components and in the intravesicular space a polynucleotide operatively encoding an antigen of the target microbe, wherein the target microbe is a virus.

25 35. A composition according to claim 34 in which the virus is selected from hepatitis B, hepatitis C, influenza and human immunodeficiency virus.

36. A composition according to claim 34 in which the polynucleotide encodes hepatitis B surface antigen or haemagglutinin.

30 37. A composition according to claim 34 which is an aqueous composition in which the liposomes are suspended in a pharmaceutically acceptable aqueous vehicle.